Eur J Vasc Endovasc Surg **28**, 71–78 (2004)
doi: 10.1016/j.ejvs.2004.03.015, available online at http://www.sciencedirect.com on SCIENCE DIRECT®

Effects of Atherosclerotic Plaque on the Enlargement of an Experimental Model of Abdominal Aortic Aneurysm in Rabbits

H. Kobayashi, M. Matsushita, K. Oda, N. Nishikimi, T. Sakurai and K. Komori*

Division of Vascular Surgery, Department of Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan

Purpose. *Abdominal aortic aneurysm (AAA) usually is associated with atherosclerosis. We attempted to create an abdominal aortic aneurysm with atherosclerotic plaque in rabbits to investigate the relationship between atherosclerosis and enlargement of AAA.*
Methods. *An isolated segment of rabbit abdominal aorta was perfused with pancreatic elastase. Animals were fed a cholesterol-enriched diet to induce atherosclerotic plaque formation. Eight animals received perfusion with elastase and were fed a cholesterol-enriched diet (group EC), eight animals received perfusion with elastase and were fed a normal diet (group EN), eight animals received perfusion with saline and were fed a cholesterol-enriched diet (group SC), and eight animals received perfusion with saline and were fed a normal diet (group SN). Four animals served as a sham group. Each animal was killed after aortography at 4 or 12 weeks. The perfused segment was excised and examined histologically.*
Results. *No animal treated with saline and fed normal diet (groups SN) developed either an aneurysm or atherosclerosis. Atherosclerotic plaque was observed in animals fed the cholesterol-enriched diet (groups SC and EC) at 4 weeks, and the plaque had thickened further by 12 weeks. All animals treated with elastase (groups EN and EC) developed an AAA. Maximum internal diameter in groups EN and EC (4.1 mm) was equal at 4 weeks, but at 12 weeks, the diameter was less for group EC than for group EN (4.0 mm versus 4.8 mm, $P < 0.05$).*
Conclusion. *Cholesterol-enriched diet following the intra-luminal perfusion of an isolated aortic segment with elastase produced an AAA with atherosclerotic plaque in rabbits. It is likely that the thickened intima with atheroma suppressed continued enlargement of the aneurysm in this model.*

Key Words: Abdominal aortic aneurysm; Experimental model; Rabbits; Atherosclerosis; Hyperlipidemia.

Introduction

Atherosclerosis usually is a prominent feature in abdominal aortic aneurysm (AAA) and accompanies destruction and functional loss of elastin in the aortic wall.[1,2] Several experimental models of aneurysm have been developed to investigate the pathogenesis of AAA. However, most of these models do not involve atherosclerosis but destroy or remove the elastic laminae only.[3–6] On the other hand, experimental atherosclerosis rarely induces AAA. Aneurysms formed in only 1% of monkeys fed a diet high in cholesterol for 12–24 months.[7]

Rabbits have been used to study atherosclerosis because they can readily be made hypercholesterolemic by feeding with an atherogenic diet.[8,9] However, there are few models of AAA in rabbits. We developed a rabbit model of AAA with pre-atherosclerotic intimal hyperplasia.[10] In that model, the aneurysm was induced by infusion of pancreatic elastase in an isolated segment of aorta, and intimal hyperplasia was developed by mechanical injury to the intima. In this report, we attempted to induce atherosclerotic plaque in an experimental model of AAA by feeding with a diet high in cholesterol. Our purpose was to investigate the relationship between atherosclerosis and the development of the AAA.

Materials and Methods

Rabbit colony

Thirty-six female Japanese-White rabbits (Nippon SLC, Hamamatsu, Japan) each weighing 1.8–2.5 kg

*Corresponding author. Kimihiro Komori, Division of Vascular Surgery, Department of Surgery, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan.

1078–5884/010071 + 08 $35.00/0

(11–13 weeks of age) were used. All experimental protocols and animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals (Washington, National Academy Press, 1996) and were approved by the Institutional Animal Care and Use Committee of the Nagoya University Graduate School of Medicine. All animals were housed in an alternating light-dark environment with diet and water freely available for the duration of the experiment.

Experimental model

Prior to operation, body weight was measured and a blood sample was obtained from a subcutaneous vein in the ear, to measure the total serum cholesterol. Body weight and total serum cholesterol were measured monthly thereafter. The rabbits were anesthetized with pentobarbital sodium (Nembutal: Abbott Laboratories, North Chicago, Ill., USA) 30–40 mg/kg given intravenously. Intermittent bolus intravenous injections of one-quarter dose of the initial anesthetic agents were administered to maintain an adequate level of anesthesia. The abdomen was approached by a midline laparotomy. Abdominal aorta and both common iliac arteries were exposed and freed from the vena cava. The abdominal aorta was exposed between the aortic bifurcation and the posterior mesenteric artery, which is about 2 cm proximal to the aortic bifurcation. Each lumbar artery was carefully exposed and preserved. The external diameter was measured in all animals just after the exposure of abdominal aorta by a single observer (H.K.). The isolated segment was catheterized via the left common iliac artery, and a 22 G cannula (Surflo IV Catheter: Terumo, Tokyo, Japan) was introduced into the aortic lumen. The left common iliac artery was tied with a silicone loop (SURG-I-LOOP: Scanlan, St Paul, MN) to encompass the cannula. An atraumatic vascular clamp was placed on the aorta just distal to the posterior mesenteric artery. The lumbar arteries, originating from the isolated segment of aorta, were clamped with disposable vascular clips (Bear Disposable Vascular Clip: Kyowa Precision Instruments Corp, Ichikawa, Japan). Physiological saline (1 ml) was injected through the cannula to flush any blood in the clamped segment into the right common iliac artery. Then the right common iliac artery was clamped with a disposable vascular clip (Bear), and any saline in the clamped segment was aspirated. The isolated segment of abdominal aorta was filled with the appropriate solution. Solutions were infused manually using gentle pressure by a single operator (M.M.), to obtain maximum expansion of the aorta. The direct pressure (about 350 mmHg) in the isolated segment was measured during the perfusion. About 150 expansions were performed for 5 min. The cannula was withdrawn after completion of the perfusion and the solution was flushed from the aortic segment to remove any remaining enzyme solution. The hole in the left common iliac artery was closed with a 6–0 polypropylene suture (Sugilene, DAVIS + GECK, Manati, PR) and the clamp was removed from the aorta to restore flow. The external diameter of the aorta was measured with a micrometer before and after perfusion during normal aortic flow by a single observer (H.K.). The abdomen was closed, and the animals then were maintained in individual cages for the duration of the experiment.

At the termination of the experiment, each animal was reanesthetized with pentobarbital sodium and a second laparotomy was performed. Contrast medium was injected into the aorta through a cannula inserted directly into the exposed infrarenal aorta, and an angiogram of the abdominal aorta was created. The size of the aorta was measured on this angiogram. The aorta was considered to be aneurysmal when the diameter was more than 150% the size of the unperfused aorta just proximal to the perfused segment.

After the arteriography, the animals were killed by a lethal overdose of pentobarbital sodium and the abdominal aortas were removed for histological examination.

Experimental design

The perfusion of the isolated segment of abdominal aorta was performed using an elastase solution or physiologic saline as control. For elastase solution, 100 U/mL of porcine pancreatic elastase (Sigma Chemical Co., St Louis, MO) was used at room temperature. Since this enzyme is the most active around at pH 9, the solution was adjusted to that pH with 0.1N NaOH, as previously reported.[10] Experimental diet was started the day following the operation. Animals were fed 1% cholesterol-enriched diet or normal rabbit chow.

The animals were divided into five groups. Group EC ($n = 8$) underwent perfusion of the isolated aorta with elastase solution and were fed the cholesterol-enriched diet. Group EN ($n = 8$) underwent perfusion of the isolated aorta with elastase solution and were fed normal diet. Group SC ($n = 8$) underwent perfusion of the isolated aorta with physiological saline and was fed the cholesterol-enriched diet. Group SN

($n = 8$) underwent perfusion of the isolated aorta with physiological saline and was fed a normal diet. Each group was divided into two subgroups of four animals each, which were killed at 4 or 12 weeks. No animals were withdrawn from the experimental protocol. Group D ($n = 4$) was the sham group. The animals of group D underwent aortic exposure and had their aortas measured and clamped only, without perfusion, and were fed the cholesterol-enriched diet for 4 weeks. Since we planned to examine the influence of saline perfusion using group SN, group D animals did not undergo aortic perfusion.

Histology

The excised aortas were fixed in 10% buffered formalin for conventional histology. One transected segment of the perfused segment of aorta, corresponding to the dilated site on arteriography, from each animal was embedded in paraffin. The sections were stained with hematoxylin and eosin, elastic van-Gieson stain for elastin, and Sudan III stain for foam cells and examined using light microscopy.

A single pathologist, blind to treatment allocation, measured the maximum thickness of the intima in all sections from each group.

Statistical methods

Statistical analysis was performed using the computer statistical package STATVIEW 5.0 (Abacus Concepts, 1998,Berkeley, Calif). Results are expressed as mean ± SD. Differences were assessed by analysis of variance (ANOVA) followed by Fisher's PLSD procedure. Statistically significant differences were accepted at the $P < 0.05$ level.

Results

Patterns of hyperlipidemia

Fig. 1 shows the average serum cholesterol concentration of the animals in each group. The initial serum cholesterol concentration for all animals was 1.24 ± 0.47 mmol/L and rose rapidly with cholesterol-enriched diet feeding. After 4 weeks of the atherogenic diet, the cholesterol concentration approached 25 mmol/L in groups SC and EC. At 12 weeks, the cholesterol concentration was 35.3 ± 18.3 mmol/L (range, 16.8–53.3 mmol/L) in group SC, 29.3 ± 7.2 mmol/L (range, 22.5–35.6 mmol/L) in group EC animals. In contrast, rabbits fed a normal diet, groups SN and EN, the serum cholesterol concentration remained below 2.59 mmol/L for the duration of the experiment.

Body weight

The body weights for each group are presented in Table 1. The initial body weight was about 2.0 kg, and

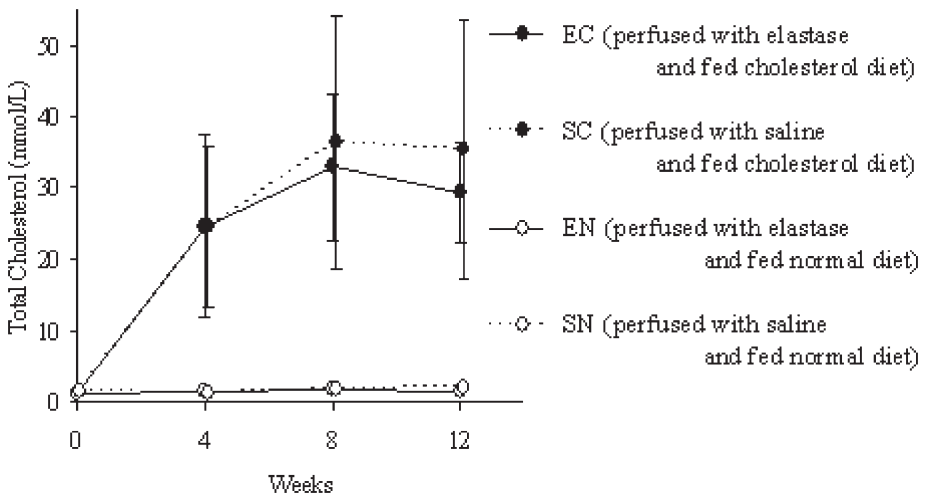

Fig. 1. Time course of the changes in total serum cholesterol concentration in each group of rabbits. (Group EC, perfused with elastase and fed cholesterol-enriched diet; Group SC, perfused with saline and fed cholesterol-enriched diet; Group EN, perfused with elastase and fed normal diet; Group SN, perfused with saline and fed normal diet; and Group D, only dissected and fed cholesterol-enriched diet). The initial mean concentration in all groups was about 1.24 mmol/L. After 4 weeks of cholesterol loading, the concentration approached 25 mmol/L in group SC and group EC. At 8 weeks the cholesterol concentration rose further and remained high subsequently in groups SC and EC. The cholesterol concentration in normal diet groups (SN and EN) remained below 2.59 mmol/L during the course of the experiment.

Table 1. Body weight in each group of rabbits

	0w	4w	12w
EC	2.04 ± 0.20	2.80 ± 0.29*	3.24 ± 0.09*
EN	2.01 ± 0.08	2.25 ± 0.06*	2.95 ± 0.23*
SC	1.98 ± 0.16	2.57 ± 0.45	3.14 ± 0.22
SN	2.01 ± 0.12	2.37 ± 0.21*	2.99 ± 0.19

*$P < 0.05$. Data are presented as mean ± SD in kg. EC, perfused with elastase and fed cholesterol diet; EN, perfused with elastase and fed normal diet; SC, perfused with saline and fed cholesterol diet; SN, perfused with saline and normal diet.

was similar in all groups. Animals in group EC had gained significantly more weight than those in group EN at 4 weeks ($P = 0.017$) and 12 weeks ($P = 0.049$). There was a significant weight difference between group EC and group SN at 4 weeks ($P = 0.039$), but not 12 weeks. The body weight was similar in the other groups both at 4 weeks and 12 weeks.

The effect of perfusion on aortic diameter

Prior to perfusion, the mean diameter of the exposed abdominal aorta was 2.39 ± 0.38 mm, and was similar in all groups. Just after perfusion, the mean aortic diameter increased to 3.47 ± 0.31 mm, but the size was still similar in saline and elastase groups. The size was unchanged for group D (Fig. 2).

Internal aortic diameter by angiography

Four weeks after perfusion, the perfused segment of aorta was aneurysmal in all animals in groups EN and EC (Fig. 3(C) and (D)), while no animal in groups SN or SC developed an aortic aneurysm, although there

Table 2. Diameter of the abdominal aorta in each group of rabbits

	4w	12w
EC	4.09 ± 0.32 (NS)*,**	3.99 ± 0.29 (NS)*
EN	4.11 ± 0.61 (NS)	4.80 ± 0.96*
SC	3.00 ± 0.08*	2.94 ± 0.13*
SN	2.80 ± 0.22**	3.20 ± 0.28 (NS)

*$P < 0.05$; **$P < 0.005$; NS, not significant. Data are presented as mean ± SD in mm. Diameters of each animal is as follows: EC—perfused with elastase and fed cholesterol diet, (4w: 4.00, 3.80, 4.55, 4.00; 12w: 3.65, 4.35, 3.95, 4.00). EN—perfused with elastase and fed normal diet, (4w: 5.00, 4.00, 3.60, 3.85; 12w: 4.05, 5.15, 6.00, 4.00). SC—perfused with saline and fed cholesterol diet, (4w: 2.90, 3.00, 3.00, 3.10; 12w: 3.00, 3.00, 3.00, 2.75). SN—perfused with saline and normal diet, (4w: 3.00, 2.80, 2.50, 2.90; 12w: 3.00, 3.60, 3.00, 3.20).

was non-aneurysmal dilation of less than 150% of the internal diameter of the unperfused aorta (Fig. 3(B)). The diameters of the abdominal aortas in each group are presented in Table 2. The internal aortic diameter was significantly greater in groups EN and EC than in the groups SN and SC at 4 weeks ($P < 0.0005$). At 12 weeks, animals in group EC had a significantly smaller internal aortic diameter than those in group EN ($P = 0.0489$). There were also significant differences between group EN and group SC ($P = 0.0003$), group EN and group SN ($P = 0.0010$), and group EC and group SC ($P = 0.0151$). The internal aortic diameter was 2.79 ± 0.10 mm at 4 weeks in the sham group (Group D) with no dilatation of the exposed segment (Fig. 3(A)).

Histologic findings

Intimal hyperplasia was evident for group SN at 4 weeks, with preservation of the internal elastic lamina

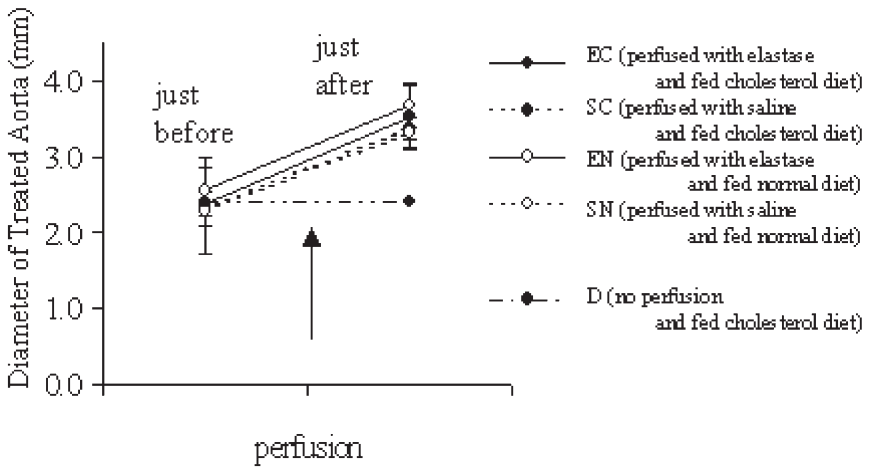

Fig. 2. The acute effect of aortic perfusion on aortic diameter. (Group EC, perfused with elastase and fed cholesterol-enriched diet; Group SC, perfused with saline and fed cholesterol-enriched diet; Group EN, perfused with elastase and fed normal diet; Group SN, perfused with saline and fed normal diet; and Group D, sham and fed cholesterol-enriched diet). The diameter increased similarly in all groups undergoing perfusion, but was unchanged in the Sham group (Group D).

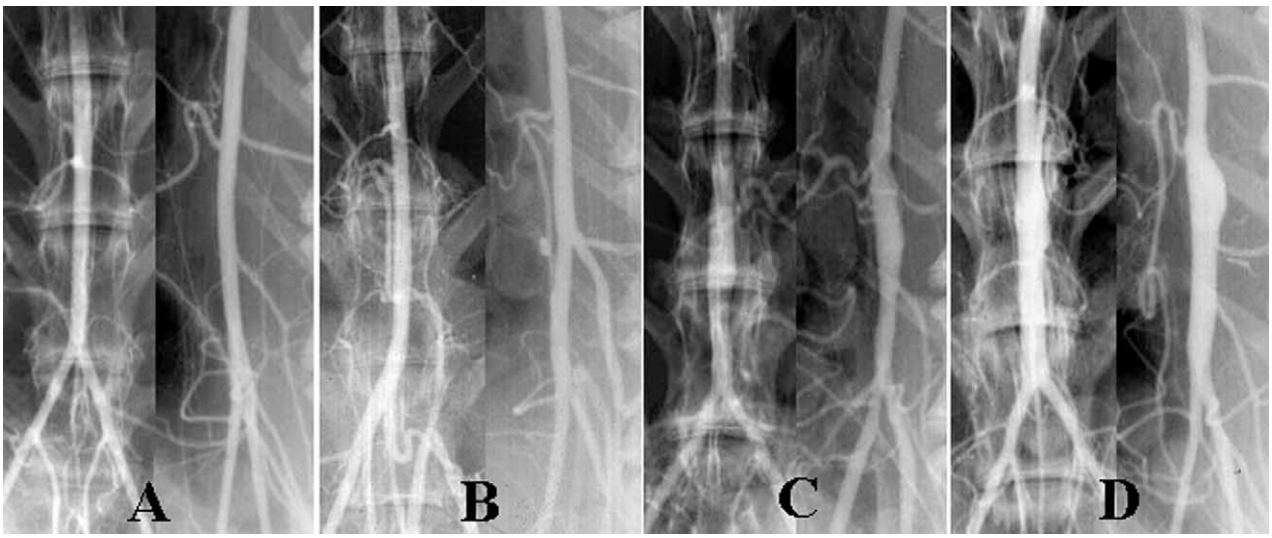

Fig. 3. Anteroposterior and lateral aortography. (A) Group D (aortic dissection and clamping only without perfusion and fed cholesterol-enriched diet for 4 weeks). The dissected segment is not dilated. (B) Group SC (the aorta perfused with saline and animal fed cholesterol-enriched diet for 12 weeks). There is no dilation in the perfused segment. (C) Group EC (the isolated segment perfused with elastase and fed cholesterol-enriched diet for 4 weeks). The perfused segment is dilated and is greater than 150% the size of the proximal aorta. (D) Group EN (the isolated segment perfused with elastase and fed normal diet for 12 weeks). An aneurysm is seen clearly below the level of the posterior mesenteric artery.

and media (Fig. 4(A)). In the aortas treated with elastase (group EC at 4 weeks), intimal thickening with atheroma formation was observed. The internal elastic lamina was fragmented, and had almost disappeared (Figs. 4(B) and 5(C)). The medial structure showed degeneration and thinning.

At 12 weeks, group SN demonstrated marked intimal hyperplasia (Fig. 5(A)). In group SC, there was thickening of the intima accompanying marked atheromatous change, and numerous foamy macrophages were seen (Fig. 5(B)). In group EN, the internal elastic lamina was partially interrupted, with thinning of the media (Fig. 5(C)). Intimal hyperplasia and marked disorganization of the elastic lamellae were noted in group EN (Fig. 5(C)) and in group EC (Fig. 5(D)). In group EC, the intima had become markedly thickened and was accompanied by atheromatous plaque containing numerous foamy macrophages.

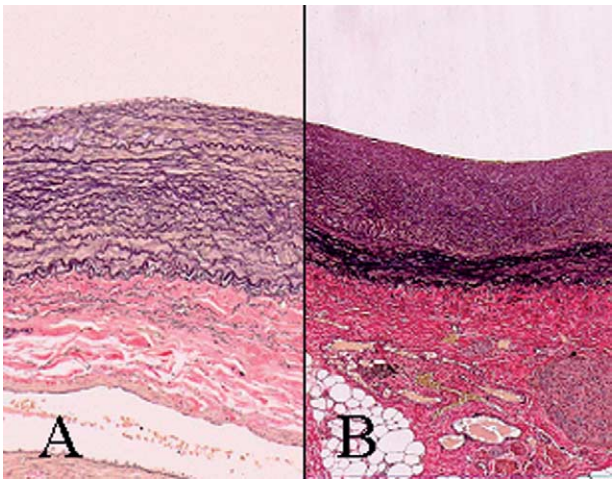

Fig. 4. Photomicrographs comparing the histology of rabbit aortas at 4 weeks. Group SN (perfused with saline and fed normal diet) and EC (perfused with elastase and fed cholesterol-enriched diet) rabbits at 4 weeks. (A) In group SN, the aorta shows intimal hyperplasia and preservation of the internal elastic lamina and media (elastic van-Gieson stain (EVG) × 130). (B) Group EC has thickened intima, with degeneration and thinning of the media. (EVG × 100).

The intima was the thickest in hyperlipidemic rabbits treated with elastase; group EC, comparing to those in group SC ($P = 0.0141$), EN ($P = 0.0141$) and SN ($P = 0.0008$) at 4 weeks after treatment (Table 3). In the hyperlipidemic rabbits, groups EC and SC, the intima continued to thicken over the next 8 weeks. Intimal thickness at 12 weeks was greater in groups EC and SC than group SN significantly ($P < 0.01$). Intimal thickness in groups EN and SN was similar at 4 weeks and 12 weeks (Table 3).

Discussion

The development of an experimental model of atheromatous aneurysm is would facilitate understanding of the human pathology,[11] and a number of experimental aneurysm models have been developed. Toxic substances, such as acetizoate, have been injected into the media to destroy it and/or into the

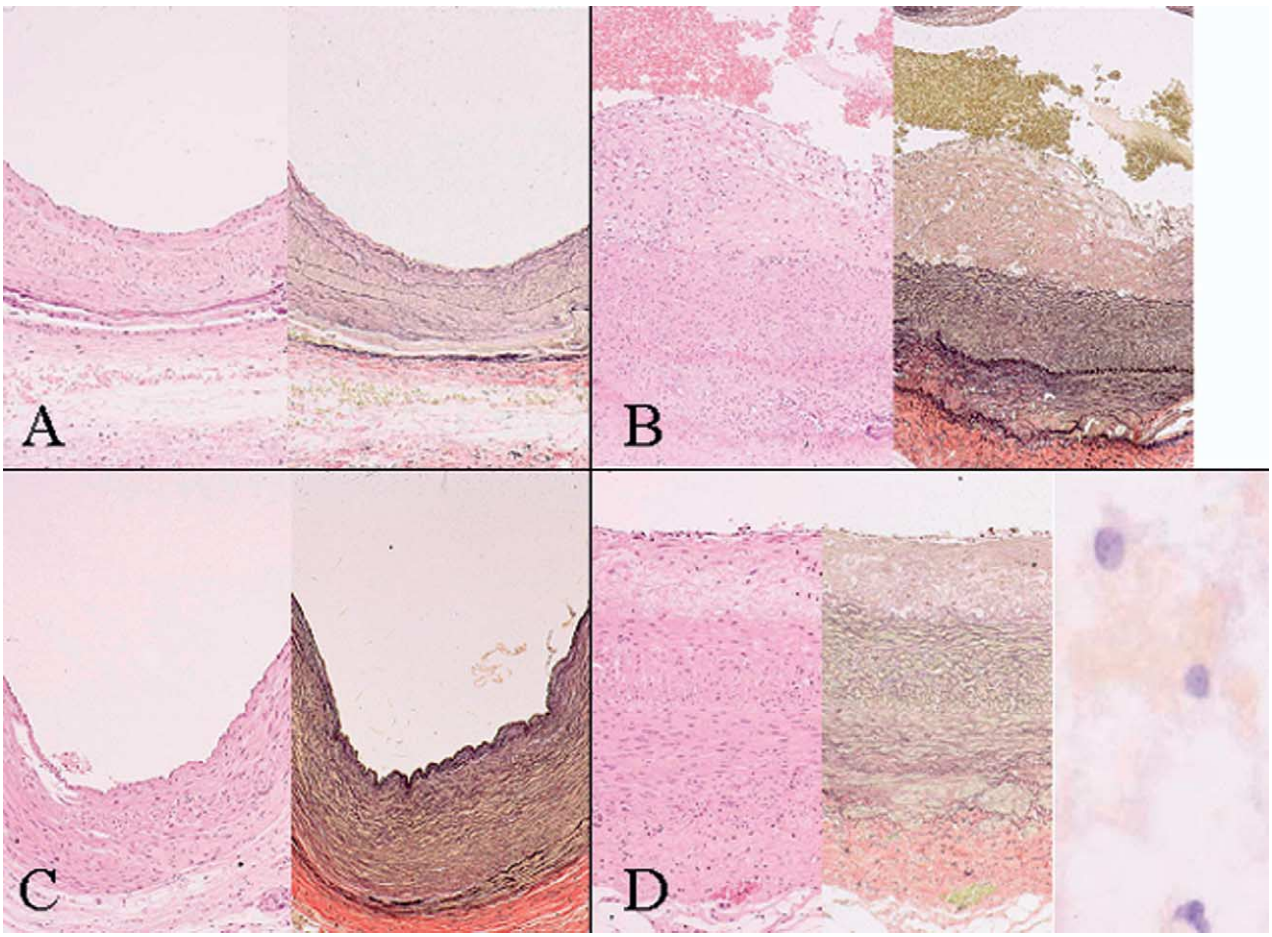

Fig. 5. Photomicrographs comparing the histology of rabbit aortas at 12 weeks. Group SN (perfused with saline and fed normal diet), SC (perfused with saline and fed cholesterol-enriched diet), EN (perfused with elastase and fed normal diet), and EC (perfused with elastase and fed cholesterol-enriched diet). (A) Group SN demonstrated marked intimal hyperplasia (left, hematoxylin and eosin (HE) × 100 and right, elastic van-Gieson stain (EVG) × 100). (B) Group SC showed thickening of the intima accompanied by marked atheromatous changes. Numerous foamy macrophages were seen in the atheroma (left, HE × 100 and right, EVG × 100). (C) In group EN, the internal elastic lamina was partially disrupted with thinning of the media (left, HE × 100 and right, EVG × 100). Intimal hyperplasia is marked, and the elastic lamellae are disorganized in both group EN (C) and in group EC (D) (left, HE × 100 and center, EVG × 100). In group EC, the intima is highly thickened and marked atheromatous change with numerous foamy macrophages is seen (D, right). (Sudan III × 1000). The internal elastic lamina is rarely observed (D, center) (EVG × 100).

Table 3. The maximum thickness of the intima in each group of rabbits

	4w	2w
EC	0.35 ± 0.12*,**	0.50 ± 0.07 (NS)*,**
EN	0.20 ± 0.09*	0.28 ± 0.03*
SC	0.20 ± 0.04*	0.45 ± 0.24 (NS)
SN	0.13 ± 0.07**	0.18 ± 0.03**

*$P < 0.05$; **$P < 0.005$; NS, not significant. Data are presented as mean ± SD in mm. EC, perfused with elastase and fed cholesterol diet; EN, perfused with elastase and fed normal diet; SC, perfused with saline and fed cholesterol diet; SN, perfused with saline and normal diet.

adventitia in dogs.[12] Arterial dilation of the rabbit common carotid artery can be produced by periarterial application of calcium chloride.[13] Perhaps the most interesting model at present are arising from studies of arterial injury by direct infusion of pancreatic elastase into an isolated segment of the rat abdominal aorta.[4] Histologically, these aneurysms are characterized by the near total destruction of the elastin matrix of the media. It appears that macrophages within the aortic media may be responsible for elastase secretion and subsequent aneurysmal degeneration.[4,14] The different models support the hypothesis that medial injury with elastolysis is necessary for aneurysm formation, and that the inflammatory response facilitates this process.

However, these experimental aneurysms are rarely accompanied by atherosclerotic intimal plaque formation, as usually observed in clinical practice.

The detailed pathogenesis of human AAA remains poorly understood. However, atherosclerosis is considered to play a contributing role, as has hyperlipidemia.[11] Glagov *et al*.[15] reported that atherosclerotic deposits are accompanied by arterial enlargement and that enlargement tends to compensate for the increase in intimal plaque area, thereby preventing or postponing the development of lumen stenosis. A positive correlation between plaque size and enlargement of the infrarenal aorta was reported in a postmortem study of male cadavers.[16] Proteolytic enzymes in the complicated plaque may digest elastic fibers in the media, resulting in aneurysmal enlargement.[16,17] Reed *et al*.[2] concluded that the risk factors (including hypertension, smoking and hyperlipidemia) for aortic atherosclerosis are necessary elements in the final common pathways and are associated with an increased incidence of AAA. The pathology is interpreted as atherosclerotic-induced medial disruption of the aneurysmal wall and atrophic degeneration with fibrous replacement of elastin and inflammation of the media and adventitia.

In this study, there was no significant difference in the aortic diameter between animals treated with elastase and those treated with saline just after perfusion. However, only those treated with elastase developed an abdominal aortic aneurysm, presumably the aneurysm was caused by the destruction of the aortic wall as a late effect of the elastase treatment. The addition of a cholesterol-enriched diet caused AAA formation with atherosclerosis. Based on histology, we believe that our model is more similar to human AAA than other available models. In human aneurysms, there are variable numbers of inflammatory cells in the adventitia, and several experimental aneurysms developed the same pattern of adventitial inflammation.[6,13] Atherosclerotic plaque did not occur in these models. Although our model differs from human abdominal aortic aneurysm with respect to adventitial inflammation, we do not believe that it is an important difference. Sections through human AAAs often show inflammation in the adventitia, which is thought, by some investigators, to be a cause of medial elastin degeneration. However, if atherosclerosis plays a role in the development of aneurysm, medial degeneration should be induced from the intimal site because atherosclerosis starts in the intima.

All animals treated with elastase perfusion had developed AAA within 4 weeks of treatment independent of their diet, and even animals fed a normal diet experienced further enlargement of the aorta over the subsequent 8 weeks. However, cholesterol-enriched diet alone did not lead to enlargement of the aorta. The histology of aortas from animals treated with elastase perfusion and fed the cholesterol-enriched diet suggests the reason for these results. Atherosclerotic plaques contained characteristic foamy cells over a thinned media and disrupted elastic lamellae. The atherosclerotic plaques continued to enlarge between 4 and 12 weeks, even though the media changed little. It is possible that the atherosclerotic plaque contributed to the structural support of the artery wall. Using an experimental model, Zarins *et al*.[7,18] also reported that progressive or stable atherosclerotic plaques overlying atrophic media provide structural reinforcement in early phases of aneurysm formation, particularly in association with the fibrogenesis and cell proliferation that characterizes plaque formation. If, late in the disease or during regression, the plaque is reduced in size and/or is altered in composition, tensile support may be insufficient and aneurysmal formation may ensue.[16,19] Freestone *et al*.[20] described a rabbit model of AAA with atherosclerosis created by periaortic injection of calcium chloride and thiogycollate in conjunction with an atherogenic diet. In that case, hypercholesterolemia correlated with rapid aortic dilation but not intimal hyperplasia. It was hypothesized that hypercholesterolemia influenced activation of moncyte chemotaxis, which led to infiltration by inflammatory cells within 4 weeks and initiated aneurysm formation. However, our findings do not support their conclusion. We believe that progression of the atherosclerotic plaque inhibits aneurysmal dilation in the early phase in the hypercholesteremic rabbit model. In the later phase, the intimal plaque itself may cause atrophy of the media with matrix metalloproteinases (MMPs) produced by large numbers of foamy cells.

Vascular remodelling is now recognized as the major factor in arterial modification after arterial injury.[21,22] Restrictive remodelling is the cause of stenosis rather than intimal hyperplasia but expansive remodelling is the more important process in aneurysm development. Considering our results in terms of vascular remodelling, after perfusion with elastase expansive remodelling was progressive, resulting in aneurysm and restrictive remodelling did not occur during the time period studied.

The histology of our model resembles that of human AAAs more closely than any other available animal model because it contains prominent atherosclerosis and degeneration of the media. Longer-term studies to examine the relationship between plaque formation and destruction and aneurysm enlargement are needed.

Acknowledgements

The authors thank Dr Masahiko Ando, Health and Medical Service, Kyoto University, who kindly provide his expertise in statistics and helped with the statistical methods and interpretation of the results.

References

1 MacSweeney ST, Powell JT, Greenhalgh RM. Pathogenesis of abdominal aortic aneurysm. *Br J Surg* 1994; **81**:935–941.
2 Reed D, Reed C, Stemmermann G, Hayashi T. Are aortic aneurysms caused by atherosclerosis? *Circulation* 1992; **85**: 205–211.
3 Freestone T, Turner RJ, Coady A, Higman DJ, Greenhalgh RM, Powell JT. Inflammation and matrix metalloproteinases in the enlarging abdominal aortic aneurysm. *Arterioscler Thromb Vasc Biol* 1995; **15**:1145–1151.
4 Anidjar S, Salzmann JL, Gentric D, Lagneau P, Camilleri JP, Michel JB. Elastase-induced experimental aneurysms in rats. *Circulation* 1990; **82**:973–981.
5 Halpern VJ, Nackman GB, Gandhi RH, Scholes JV, Ramey WG, Tilson MD. The elastase infusion model of experimental aortic aneurysms: synchrony of induction of endogenous proteinases with matrix destruction and inflammatory cell response. *J Vasc Surg* 1994; **20**:51–60.
6 Anidjar S, Dobrin PB, Eichorst M, Graham GP, Chejifec G. Correction of inflammatory infiltrate with the enlargement of experimental aortic aneurysms. *J Vasc Surg* 1992; **16**:139–147.
7 Zarins CK, Glagvo S, Vesselinovitch D, Wissler RW. Aneurysm formation in experimental atherosclerosis: relationship to plaque evolution. *J Vasc Surg* 1990; **12**:246–256.
8 Poole JCF, Florey HW. Changes in the endothelium of the aorta and the behavior of macrophages in experimental atheroma in rabbits. *J Pathol Bacteriol* 1958; **75**:245–250.
9 Imai H, Lee KT, Pastan S, Pamilio E, Florentin R, Thomas WA. Atherosclerosis in rabbits-architectural and subcellular alteration of smooth muscle cells of aortas in response to hyperlipemia. *Exp Mol Pathol* 1966; **5**:273–310.
10 Matsushita M, Kobayashi H, Oda K, Nishikimi N, Sakurai T, Nimura N. A rabbit model of abdominal aortic aneurysm associated with intimal thickening. *Eur Surg Res* 1999; **31**: 305–313.
11 Powell JT. Models of aneurysm for the investigation of pathogenesis and pharmacotherapy: a review. *Atherosclerosis* 1991; **87**:93–102.
12 Economou SG, Taylor CB, Beattie EJ, Davis CB. Persistent experimental aortic aneurysm in dogs. *Surgery* 1960; **47**:21.
13 Gertz SD, Kurgan A, Eisenberg D. Aneurysm of rabbit common carotid artery induced by periarterial application of calcium chloride *in vivo*. *J Clin Invest* 1988; **81**:649–656.
14 Anidjar S, Dobrin PB, Chejfec G. Progressive enlargement of experimental aortic aneurysm is associated with infiltration of inflammatory cells. *J Cardiovasc Surg* 1991; **32**:39–40.
15 Glagov S, Weisenberg E, Kolettis G, Stankunavicius R, Zarins CK. Compensatory enlargement of human atherosclerotic coronary arteries. *N Engl J Med* 1987; **316**:1371–1375.
16 Xu C, Zarins CK, Glagov S. Aneurysmal and occlusive atherosclerosis of the human abdominal aorta. *J Vasc Surg* 2001; **33**:91–96.
17 Matsushita M, Nishimimi N, Sakurai T, Nimura T. Infrarenal aortic dilatation less than 4 cm is not unusual in patients with aortoiliac occlusive disease. *Int Angiol* 2002; **21**:222–227.
18 Zarins CK, Xu C, Glagov S. Aneurysmal enlargement of the aorta during regression of experimental atherosclerosis. *J Vasc Surg* 1992; **15**:90–101.
19 Zarins CK, Xu C, Glagov S. Atherosclerotic enlargement of human abdominal aorta. *Atherosclerosis* 2001; **155**:157–164.
20 Freestone T, Turner RJ, Higman DJ, Lever MJ, Powell JT. Influence of hypercholesterolemia and adventitial inflammation on the development of aortic aneurysm in rabbits. *Arterioscler Thromb Vasc Biol* 1997; **17**:10–17.
21 Pasterkamp G, Smits PC. Imaging atherosclerosis. Remodelling of coronary arteries. *J Cardiovasc Risk* 2002; **9**:229–235.
22 Vink A, de Kleijn DP, Borst C, Pasterkamp G. Remodelling of arterial wall: a determinant of luminal stenosis and a factor with prognostic significance. *Ned Tijdschr Geneeskd* 2002; **146**: 1264–1270.

Accepted 16 March 2004
Available online 14 April 2004